PATENT

Attorney Docket No.: MKC-008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Lockyer	CONF. NO.:	1674
SERIAL NO.:	10/560,956	GROUP NO.:	1652
FILING DATE:	July 24, 2006	EXAMINER:	S. Swope
TITLE:	METHODS FOR IDENTIFYING COMPOUNDS INTERACTING WITH SMALL MEMBRANE-BOUND GTP-ASES		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

This paper is being filed in response to the Restriction Requirement mailed from the Office on March 14, 2008. Applicant encloses a Petition for Extension of Time under 37 C.F.R. § 1.136(a) extending the deadline for response by one month up to and including May 14, 2008. Applicant has charged the associated fees to Deposit Account No. 07-1700 (under Ref. No. MKC-008). Applicant believes that no other fees are required. However, please consider this a conditional petition and charge any other fees necessary for entry of this paper to Deposit Account No. 07-1700 (under Ref. No. MKC-008).

The Office has required Applicant, pursuant to 35 U.S.C. § 121, to elect a single invention for prosecution on the merits from subject matter that the Office identifies in three groups, Groups I(A)-(H), Groups I(I)-(L), and Groups I(M)-(N).

Applicant hereby elects, with traverse, the following subject matter. In Groups I(A)-(H), Applicant elects, with traverse, Group I(A), namely, the subject matter that the Office identifies as *H-Ras*. In Groups I(I)-(L), Applicant elects, with traverse, Group I(I), namely, the subject matter that the Office identifies as *Wild-type GTPase*. In Groups I(M)-(N), Applicant elects, with traverse, Group I(M), namely, the subject matter that the Office identifies as *Low throughput*.

Applicant respectfully submits that Mochizuki *et al.* (2001) "Spatio-temporal images of growth factor induced activation of Ras and Rap1," *Nature* 411: 1065-1068 (hereinafter "Mochizuki") fails to teach or describe special technical features shared by independent claims 60 and 77, and claims depending therefrom. Accordingly, Applicant requests reconsideration and withdrawal of the Restriction Requirement.

Mochizuki describes a fluorescent resonance energy transfer (FRET) assay for growth factor induced activation of Ras and Rap1. The Ras reporter, Raichu-Ras, (Raichu standing for Ras and interacting protein chimeric unit) has been engineered as a single chimeric protein that consists of a terminal yellow fluorescent protein (YFP) and a terminal cyan fluorescent protein (CFP) flanking a peptide consisting of H-Ras and the Ras-binding domain (RBD) of Raf. FRET involves the transfer of energy from a donor fluorescent molecule to an acceptor, which then emits its own fluorescence. Importantly, this process only occurs when the two fluorescent proteins are very close to one another. In serum-starved, unstimulated cells Ras is inactive, the fluorescent proteins are widely spaced apart, and the emission profile of CFP peaks at 475 nm upon excitation at 433 nm (close to the excitation maxima of CFP). On cellular stimulation Ras is activated by becoming GTP-bound, which induces Raichu-Ras to change conformation as a result of the RBD interacting with GTP-Ras. The two fluorophores are now in close proximity, so the energy that is emitted by CFP is partially captured by YFP, which emits light at 527 nm. Using computer-enhanced time-lapse video microscopy the ratio of emission at 527 nm and 475 nm can be calculated in order for the spatio-temporal dynamics of Ras activation to be measured. Similar intramolecular FRET probes have been developed for Rap and Rho family members.

Applicant's invention does not relate to a FRET-based assay. Rather, it involves translocation of a reporter from the cytosol to associate with or dissociate from a small membrane bound GTPase of interest. The reporter has a detectable marker and a small GTPase binding moiety, the latter being an effector of the small GTPase or derived therefrom (e.g. a Raf 1 RBD or derivative thereof).

Mochizuki fails to teach or suggest monitoring the association of a reporter with a membrane bound small GTPase or a membrane bound active Ras, as required by Applicant's independent claims 60 and 77, respectively, and claims depending therefrom. First, Mochizuki does not monitor a membrane bound *small GTPase* or a membrane bound *active Ras*, as required

by Applicant's independent claims 60 and 77, respectively, and claims depending therefrom. Rather, Mochizuki monitors a Raichu-Ras, which is a chimeric protein consisting of H-Ras, RBD, YFP, and CFP. (Mochizuki, page 1065, second paragraph.) Accordingly, Mochizuki monitors a chimera and does not teach or suggest monitoring an actual *small GTPase* or *active Ras*.

Second, Mochizuki fails to teach or suggest *monitoring the association of a reporter* with a membrane bound small GTPase or a membrane bound active Ras, or that *dissociation of the reporter from the membrane bound small GTPase or from the membrane bound active Ras* indicates that a test compound is capable of promoting deactivation of the GTPase or active Ras, as required by Applicant's independent claims 60 and 77, respectively, and claims depending therefrom. Rather, activation of Mochizuki's Raichu-Ras chimera causes the H-Ras element to bind to the RBD element. This alters the proximity between the two FRET fluorescent protein elements at either end of the chimera and thereby modulates fluorescence emission from the chimera. Accordingly, Mochizuki's detection arises from a conformational change in the chimera, and not from the association or dissociation of a reporter.

Moreover, since Mochizuki's FRET assay uses a single Ras-chimera, it only measures intrinsic activity of that chimera. It does not measure actual activity of endogenous Ras. Conversely, Applicant's claimed invention can measure the actual activity of various endogenous small GTPases inside a cell, for example, a tumor cell expressing oncogenic Ras or hyperactive normal Ras.

Accordingly, since Mochizuki's method fails to teach or suggest any of these technical features required by Applicant's claimed invention, Applicant respectfully requests reconsideration and withdrawal of the Restriction Requirement. If the Restriction Requirement is maintained, Applicant reserves the right to pursue any non-elected claims in one or more related applications. Applicant believes that claims 60-77, and 80 read on the aforesaid elections.

CONCLUSION

Applicant respectfully requests that the application now proceed promptly to examination. The Examiner is invited to contact the undersigned with any questions about this paper. Early favorable action is respectfully solicited.

Respectfully submitted,

Date: May 13, 2008
Reg. No. 58,312

/Randall D. Morin/

Randall D. Morin
Attorney for Applicants
Goodwin Procter LLP
Exchange Place
Boston, Massachusetts 02109
Goodwin Customer No. 051414

Tel. No.: (617) 570-1657
Fax No.: (617) 523-1231